or accidental spraying with 2,4-D until the role of 2,4-D in the accumulation of toxic quantities of nitrates in these species has been more fully determined.

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An Analysis of the Enzyme Activity of the Conditioned Salivary Response in Human Subjects

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In the early work on conditioning, it was generally assumed that the conditioned response was identical with the original or unconditioned response, both in a qualitative and a quantitative sense. Later, many workers, including Pavlov, found that the conditioned response did differ in a quantitative way from the original response. As a rule, it was found to be less vigorous (motor responses) or associated with a decreased amount of saliva (glandular response). The aim of the present experiment was to determine whether there is any chemical difference in the saliva of human subjects between the original and the conditioned response.

Eleven female college students were used in the experiment. Four specimens of saliva were taken from each subject: previous to the experiment, during the response to a bell before conditioning, during the presentation of food, and after the process of conditioning had been completed. The saliva was collected from the sublingual spaces by means of a glass pipette, so that the secretion from all the salivary glands would be represented.

The tests were made in an isolated room, with the blinds drawn to eliminate gross nonexperimental stimuli. Each subject was tested individually during a single session of about 3 hr. The unconditioned stimulus was a Cryst-O-Mint candy wafer, which induced a free flow of saliva and was neutral with respect to the chemical techniques later applied. The conditioned stimulus was an electric bell. The subjects were instructed not to eat or smoke for 4 hr previous to the series of tests and reported that they had adhered to this schedule.

The bell preceded the wafer by 10 sec, and the latter was held on the tongue for 20 sec during each trial, following the suggestion by Razran (*1*). The paired stimuli (bell–wafer) were given at short, irregular intervals, so that the time interval itself could not operate as a conditioned stimulus. The time interval between presentations of the pair ranged from 30 to 90 sec, and the series of presentations was randomized. The conditioning phase consisted of 150 paired presentations.

At ½-hr intervals, each subject was asked to report any change in the amount of the salivary secretion noticed. The 11 subjects utilized in this experiment reported a definite increase in saliva from the first interval onward.

The amount of amylase in the saliva was measured for each condition. This substance was chosen because it is the starch-hydrolyzing enzyme and the most active component of saliva (*2*). The results are reported in units of amylase activity per ml of saliva. One unit of amylase may be regarded as the amount required to digest 5 ml of 1% soluble starch to the achromic point in 10 min under the conditions of the standard analysis as presented by Hawk, Oser, and Summerson (*3*). The analytical reagents employed were: (1) light-yellow aqueous iodine solution, (2) 1% aqueous solution of soluble starch, (3) 1% aqueous solution sodium chloride, and (4) phosphate buffer ($K_2HPO_4 + KH_2PO_4$) adjusted to pH 6.6.

The results of this experiment are: (1) With respect to amylase activity, there is a statistically significant quantitative difference between the salivary secretion in response to a conditioned stimulus and the reflex response. (2) There is more amylase activity in the salivary component during the conditioned response phase than in the unconditioned response. There is a mean gain of 31.7 in units of amylase activity in the salivary conditioned response over that of the unconditioned response. This difference is significant at the 0.01 level of confidence. (3) The amylase activity in the determination of experimental conditions (1) and (2) is consistently close.

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Spectrophotometric Assay of Ascorbic Acid with Peri-Naphthindanetrione Hydrate

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When peri-naphthindanetrione hydrate (I) (*1, 2*) is allowed to react with ascorbic acid (II), the reaction is of a reddish color owing to the formation of dihydroxy-peri-naphthindone (III) (*3*). This reaction is an oxidation reduction system in which the stage of oxidation stops at the point of the formation of dehydroascorbic acid (IV).